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Vibrio cholerae PCR Detection Kit — Product Insert

Product # 38500

Vibrio cholerae is a comma-shaped, gram-negative bacterium. It is the cause of cholera in humans, which affects the upper small intestine. Transmission of the disease is mainly through contaminated food or water. Human subjects affected by cholera exhibit severe watery diarrhea and vomiting, caused by the cholera toxin produced by the bacterium. Many cases of cholera are life-threatening, as diarrhea and associated vomiting can lead to rapid dehydration and electrolyte loss. Even with the extensive research on its epidemiology, cholera still affects over 5 million people per year worldwide.

Principle of the Test and Product Description

Norgen's *Vibrio cholerae* PCR Detection Kit constituents a ready-to-use system for the isolation and the detection of *V. cholerae* using end-point PCR without enrichment. The kit first allows for the isolation of bacterial DNA from patient's stool sample using spin-column chromatography based on Norgen's proprietary resin. The DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for *V. cholerae* detection using the provided *V. cholerae* Master Mix. The *V. cholerae* Master Mix contains reagents and enzymes for the specific amplification of a 333 bp region of the *V. cholerae* genome. In addition, Norgen's *V. cholerae* PCR Detection Kit contains a second Master Mix, the PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the provided *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Lysis Solution	30 mL
Lysis Additive	3 mL
Binding Solution	6 mL
Wash Solution I	15 mL
Wash Solution II	19.5 mL
Elution Buffer	3 mL
Bead Tube	24
Mini Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
V. cholerae 2x PCR Master Mix	0.35 mL
Control 2x PCR Master Mix	0.35 mL
Isolation Control (IsoC)*[a]	0.3 mL
V. cholerae Positive Control (PosC)*[b]	0.1 mL
Nuclease Free-Water	1.25 mL
Norgen's DNA Marker	0.1 mL
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* *IsoC = Isolation Control ; PosC= Positive Control*
[a] *The positive control is cloned V. cholerae DNA fragments.*
[b] *The isolation control is a cloned PCR product.*

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Flat bed vortex or bead beater equipment
- 95-100% ethanol
- 70% ethanol

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25^{o}C). Buffers can be stored for up to 1 year without showing any reduction in performance.

The *V. cholerae* 2x PCR Master Mix, Control 2X PCR Mastermix, Isolation Control (IsoC), and *V. cholerae* Positive Control (PosC) should be kept tightly sealed and stored at -20^{o}C. These can be stored for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) of these reagents should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions

The user should exercise the following precautions when using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *Vibrio cholerae* PCR Detection Kit, including the *V. cholerae* 2x PCR Master Mix, Control 2X PCR Mastermix, Isolation Control (IsoC), and *V. cholerae* Positive Control (PosC) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's *V. cholerae* PCR Detection Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Biosafety level 2 practices are recommended for works involving *Vibrio cholerae*. Ensure the appropriate containment equipment and facilities are used for activities involving cultures or potentially infectious clinical materials. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The **Wash Solution I** contain guanidine salts, and should be handled with care. Guanidine salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Protocol

A. *Vibrio cholerae* Genomic DNA Isolation

Precaution: All samples must be treated as potentially infectious material.

Important Notes Prior to Beginning Protocol:

- All centrifugation steps are carried out in a benchtop microcentrifuge at **14,000 x g (~ 14,000 RPM)** except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of **Wash Solution II** by adding 10.5 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution II**. This will give a final volume of 30 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Isolation Control (*IsoC*)**
  - An Isolation Control (*IsoC*) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the Isolation Control (IsoC) to the lysate during the isolation procedure
  - The Isolation Control (*IsoC)* must not be added to the sample material directly.
  - Do not freeze and thaw the Isolation Control (IsoC) more than 2 times.
  - The Isolation Control (IsoC) must be kept on ice at all times during the isolation procedure.
- The PCR components of the *Vibrio cholerae* PCR Detection Kit should remain at -20^{o}C until DNA is extracted and ready for PCR amplification.

1. Lysate Preparation

a. Add up to 200 mg of stool sample to a provided Bead Tube and add 1 mL of **Lysis Solution**. Vortex briefly to mix stool and Lysis Solution.
b. Add 100 μL of Lysis Additive and vortex briefly.
c. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. Scientific Industries' Disruptor Genie^{TM}). Vortex for 3 minute at maximum speed.
d. Centrifuge the tube for 2 minute at **14000 × g (~14,000 RPM)**.
e. Transfer up to 600 μL of supernatant to a DNAase-free microcentrifuge tube (not provided).
f. Add 200 μL of Binding Solution, mix by inverting the tube a few times, and incubate for 10 minutes on ice.
g. Spin the lysate for 2 minutes to pellet any cell debris.
h. Using a pipette, transfer up to 700 μL of supernatant (avoid contacting the pellet with the pipette tip) into a 2 mL DNAase-free microcentrifuge tube (not provided).
i. Add 10 μL of **Isolation Control (*IsoC)*** to the lysis mixture, and mix by vortexing.

Note: Ensure that the **Isolation Contro (*IsoC)*** is added for subsequent control detection in the PCR protocol

j. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 μL of ethanol is added to every 100 μL of lysate). Vortex to mix. **Proceed to Step 2.**

2. Binding to Column

a. Assemble a spin column with one of the provided collection tubes.
b. Apply 600 μL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **14000 × g (~14,000 RPM**). Discard the flowthrough and reassemble the spin column with the collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

c. Repeat step **2b** with the remaining volume of lysate mixture.

3. Column Wash

a. Apply 500 μL of **Wash Solution I** to the column and centrifuge for 1 minute.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 500 μL of **Wash Solution II** to the column and centrifuge for 1 minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat **3c** and **3d**.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 μL of **Elution Buffer** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **14,000 x g (~14,000 RPM)** for 1 additional minute.

5. Storage of DNA

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

B. *Vibrio cholerae* PCR Assay Preparation

Notes:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of ***V. cholerae* 2X PCR Master Mix** and **Control 2X PCR Master Mix** provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR) each.
- For each sample, one PCR reaction using the ***V. cholerae* 2X PCR Master Mix** and one PCR reaction using **Control 2X PCR Master Mix** should be set up in order to have a proper interpretation of the result.
- For every PCR run, one reaction containing *V. cholerae* Positive Control (***PosC***) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of *V. cholerae* Limit of Detection.

1. Prepare the PCR for sample detection (Set #1, using ***V. cholerae* 2X PCR Master Mix**) and control detection (Set #2, using **Conrtol 2X PCR Master Mix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one *V. cholerae* detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. PCR Assay Preparation

PCR Components	Volume Per PCR Reaction
***V. cholerae* 2X PCR Master Mix** **Or** **Control 2X PCR Master Mix**	**10 µL**
Sample DNA	**2.5 µL**
Nuclease-Free Water	**7.5 µL**
Total Volume	**20 µL**

2. For each PCR run, prepare **one** positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

PCR Components	Volume Per RT- PCR Reaction
***V. cholerae* 2X PCR Master Mix** **Or** **Control 2X PCR Master Mix**	**10 µL**
Positive Control (PosC)	**10 µL**
Total Volume	**20 µL**

3. For each PCR run, prepare **one** no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

PCR Components	Volume Per PCR Reaction
***V. cholerae* 2X PCR Master Mix** **Or** **Control 2X PCR Master Mix**	**10 µL**
Nuclease-Free Water	**10 µL**
Total Volume	**20 µL**

Therefore, at a minimum, each PCR run will contain 6 separate PCR reactions

C. PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run PCR.

Table 4. *V. cholerae* Assay Program

One Step PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**5 min**
Cycle 2 (35x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

D. *Vibrio cholerae* PCR Assay Results Interpretation

1. For the analysis of the PCR data, the entire 15-20 µL PCR Reaction should be loaded on a 1X TAE 1.7% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided). Prepare enough agarose gel for running one set of PCR of *V. cholerae* detection and one set of PCR for controls detection.
2. The PCR products should be resolved on the 1X TAE 1.7% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

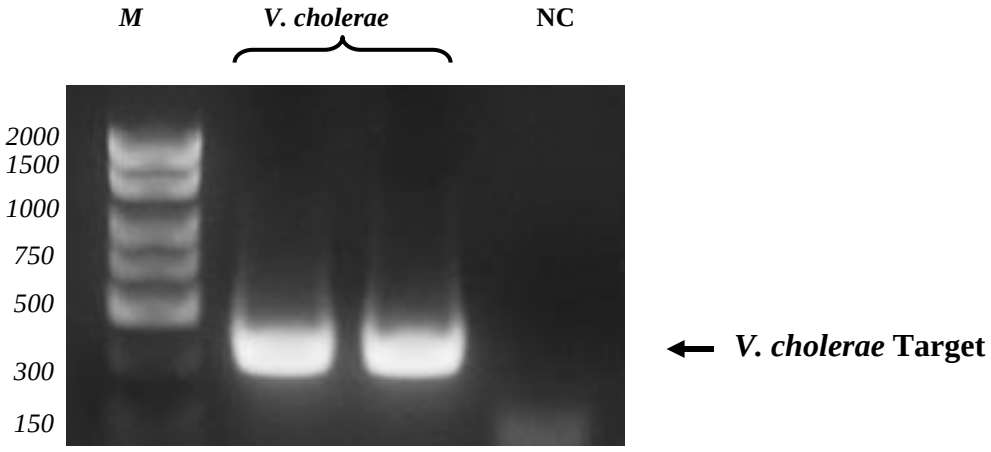

Figure 1: A representative 1X TAE 1.7% agarose gel showing the amplification of *V. cholerae* under different concentration (***V. cholerae*** Target) using the ***V. cholerae* 2X PCR Master Mix**. The size of the *V. cholerae* target amplicon corresponds to 333 bp as represented by the provided DNA Marker (M). **NC** = Negative Control.

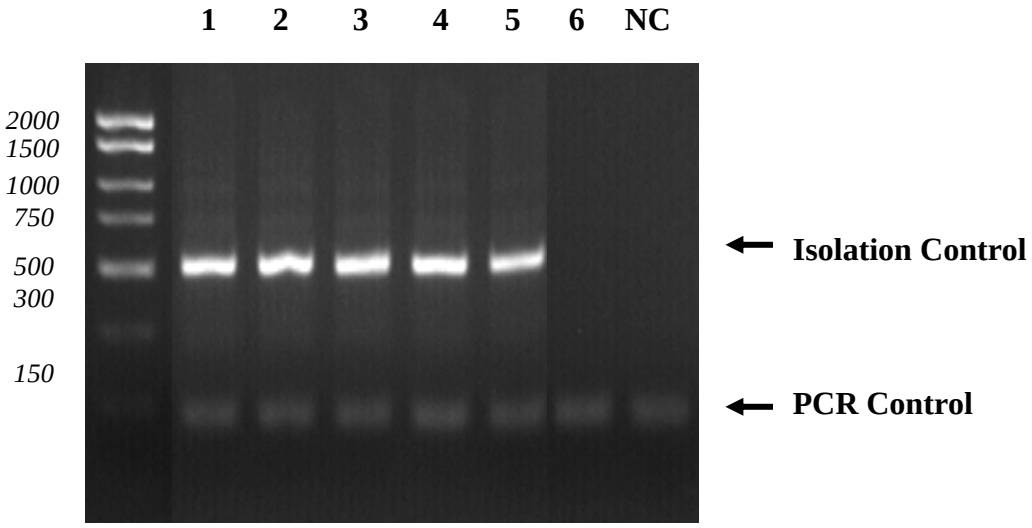

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **Control 2X PCR Master Mix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the DNA isolation as well as the PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the PCR was successful, the isolation failed to recover even the spiked-in Isolation control. **NC** = Negative Control.

Table 5. Interpretation of PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	***V. cholerae* Target Band (333 bp)**	***V. cholerae IsoC* Band (499 bp)**	***V. cholerae PCRC* Band (150 bp)**	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

****** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

E. *Vibrio cholerae* PCR Assay Specificity and Sensitivity

- The specificity of Norgen's *Vibrio cholerae* PCR Detection Kit is first and foremost ensured by the selection of the *V. cholerae*-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies to all GenBank published sequences by sequence comparison analysis. The specific detectability of all relevant strains has thus been ensured by a database alignment and by PCR amplification with the following common foodbourne disease-causing bacteria:
    - *E coli*
    - *Listeria monocytogenes*
    - *Streptococcus agalatiae*
    - *Streptococcus dysgalatiae*
    - *Staphylococcus aureus.*
    - *Salmonella sp.*

F. Linear Range

- The linear range (analytical measurement) of Norgen's *Vibrio cholerae* PCR Detection Kit was determined by analysing a dilution series of a *V. cholerae* quantification standard ranging from 1 x 10^7 cfu/µl to 1 x 10^{-1} cfu/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's *Vibrio cholerae* PCR Detection Kit on 1X TAE 1.7% Agarose gel.
- The linear range of Norgen's Vibrio cholerae PCR Detection Kit has been determined to cover concentrations from 1 x 10^2 cfu/µl to at least 1 x 10^6 cfu/µl of isolated DNA

Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's *Vibrio cholerae* PCR Detection Kitis designed to test 24 samples. For every 6 samples, a non-template control and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. How can I interpret my results if neither the PCR control (PCRC) nor the Isolation Control (IsoC) amplifies?

- If neither the PCR control nor the Isolation Control amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the Problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the PCR control (PCRC) showed amplification but neither the *V. cholerae* target nor the Isolation Control (IsoC) amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the Isolation Control (IsoC) was amplified in a sample?

- The sample tested can be considered as *V. cholerae* negative.

5. How should it be interpreted if only the *V. cholerae* target and the PCR control (PCRC) were amplified in a sample?

- The sample tested can be considered as *V. cholerae* positive.

6. How should it be interpreted if only the *V. cholerae* target was amplified in a sample?

- The sample tested should be considered as *V. cholerae* positive. At high *V. cholerae* cell input, the *V. cholerae* amplicon will be predominant and thus the PCR control (PCRC) as well as the Isolation Control (IsoC) may not amplify as they compete for PCR resources.

7. How should it be interpreted if only the PCR control (PCRC) and the Isolation Control (IsoC) showed amplification in a sample?

- The sample tested can be considered negative

8. What If I forgot to do a dry spin after my second wash?

- Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

9. What If I forgot to add Isolation Control (IsoC) during the Isolation?

- It is recommended that the isolation is repeated.

Reference

Matson JS, Withey JH and DiRita VJ. 2007. Regulatory Networks Controlling *Vibrio cholerae* Virulence Gene Expression. Infection and Immunity **75**: 5542-5549.

Related Products	Product #
Stool DNA Isolation Kit	27600
Bacterial Genomic DNA Isolation Kit	17900

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's *Vibrio cholerae* PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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